Express Mail Label No.: EV829089995US
Date of Deposit: September 21, 2006

Attorney Docket No.: 34685-502
(formerly 21882-513 UTIL (NN-13))

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Spalding and Frisen	CONF. NO.:	5366
SERIAL NUMBER:	10/654,669	EXAMINER:	Angela Bertagna
FILING DATE:	September 3, 2003	ART UNIT:	1637
FOR:	**METHOD FOR RETROSPECTIVE BIRTH DATING OF BIOMOLECULES, CELLS, TISSUES, ORGANS AND ORGANISMS**		

MAIL STOP AF
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. JONAS FRISEN UNDER 37 C.F.R. § 1.132

I, JONAS FRISEN, declare and state that:

1. I received a Medical Degree from The Karolinska Institute, Sweden in 1991, and a Ph.D. degree in Medicine from The Karolinska Institute, Sweden in 1993. I finished postdoctoral training in Dr. Mariano Barbacid's laboratory, Dept. of Molecular Biology, Bristol-Myers Squibb, Princeton, New Jersey, USA in 1997.
2. I am currently employed as a Professor of Stem Cells in the Department of Cell and Molecular Biology, at The Karolinska Institute, Sweden.
3. I am a co-inventor, together with Dr. Kirsty Spalding, of the subject matter claimed in the above referenced U.S. patent application.
4. I have reviewed the instant application, the June 6, 2006 Office Action, and the Wild (Wild et al., Radiocarbon 40:273-281, (1998)) and Hedges (Hedges et al., Radiocarbon 37: 285-290 (1995)) references.
5. I understand that the Examiner takes the position that the pending claims, claims 17, 21-23, 32 and 33, are obvious in view of a combination of Wild and Hedges.

6. I make this declaration to address the Examiner's concern. In particular, I explain below that the claimed invention would not be obvious to one of skill in the art, at the time of filing, based upon the disclosures of Wild, Hedges, or a combination of Wild and Hedges.

7. Based on information and belief, it is my opinion that the claimed "birth dating" method is the first disclosure of a reliable method for determining the age of birth of an animal. The claimed invention is novel and nonobvious over other dating methods known at the time of filing, including Wild, Hedges, or a combination of Wild and Hedges, allows the determination of a "birth date" of any animal - which is the subject matter of the instant pending claims.

8. One fundamental principal of the present invention is the measurement of a "birth date" using the tooth enamel of an organism. This is discussed throughout the originally filed application - particularly in the section entitled "Example 3" on pages 23 and 24.

9. This determination of a "birth date" by analyzing tooth enamel is not possible with the methods of Wild, Hedges, or a combination of Wild and Hedges. That is, a combination of Wild and Hedges does not teach or suggest a method to determine birth dates.

10. The claimed method of birth dating involves collecting a sample of tooth enamel from an animal which is purified away from other carbon containing molecules of said animal, determining a delta ^{14}C value of the carbon atoms in said tooth enamel; and comparing the delta ^{14}C value with a calibration delta ^{14}C chart and determining a birth date of said tooth enamel. Importantly, the method of the claimed invention can determine a birth date of an animal to within 5 years of its actual birth date. This method is recited explicitly in claim 17 and incorporated by dependency into every pending claim of the instant application.

11. Numerous advantages are achieved with the present invention. Birth dating may be carried out with small amount of tooth enamel - even if the sample is

substantially degraded and other organic matter, such as lipids and collagen are missing.

12. The current methods of ^{14}C dating, as exemplified by Wild and Hedges, cannot provide information for determining a "birth date" of an organism to within 5 years, as claimed in the instant claims. Since ^{14}C is in constant turnover, current methods of ^{14}C dating, at best, can only determine the date of death of an organism - when the organism stops incorporating new ^{14}C. Since an animal, such as a human, can have a lifespan of 10, 50 or even 100 years, the date of death provides almost no information of the birth date of an animal and cannot provide information of the birth date of an animal to within 5 years as claimed in the present claims.

13. I believe that a person of skill in the art would not find a motivation to combine the disclosures of Wild and Hedges for the reasons stated below. I also believe that the combination of Wild and Hedges cannot be made because it would be inoperable. Further, I believe that even if Wild and Hedges were combined (for the sake of argument since I believe that they cannot be combined to become a functioning method) they would not lead to the claimed method.

14. Wild is directed to the measurement of "the time of death" by measuring organic components with a rapid turnover rate. See, for example, Wild's abstract which states "[w]e also studied the applicability of the ^{14}C method in forensic sciences to determine the time of death of human individuals" (emphasis added). Wild also stated "the aim of this investigation was to find organic components of the human body with a rapid turnover time, so that measuring the $^{14}C/^{12}C$ ratios of their organic fractions and comparison of the derived values with the atmospheric "bomb" peak would lead to an accurate estimate of the time of death of the human individual. (Wild, page 274, first partial paragraph). To determine the age of bones, Wild extracted lipids from bone and bone marrow for study. See, Wild, page 274, 4th full paragraph. Thus, it is clear that Wild is directed to finding organic components of the human body with a rapid turnover time, such as lipid, so that the time of death

may be accurately determined. See, also, Wild, page 280 which states that "From our results it can be concluded that for the determination of the time of death of humans - often an important question in forensic investigations - application of the ^{14}C method yields good estimates, provided that lipids from bones or from bone marrow are available. In case of advanced decomposition, where lipids are already degraded, hair is a good alternative for such investigation" (emphasis added). Thus, it is clear that Wild depends on the use of lipids from bone or marrow and that Wild's method would not work if the lipids were decayed. Wild does not contemplate determining "birth dates" because measurement of rapid turnover molecules cannot be used to determine birth dates. ^{14}C dating is based on detecting ^{14}C levels of biological samples after the sample has ceased to incorporate additional ^{14}C. For a rapid turnover molecule, such as the molecules Wild uses for his methods, the molecule only ceases to incorporate ^{14}C after the death of the animal. An analysis of a rapidly turnover molecule can only determine a date of death.

15. Since Wild is directed to rapid turnover molecules, Wild does not suggest the use of tooth enamel as is claimed in the instant pending claims. In fact the use of tooth enamel is not suggested or disclosed by Wild.

16. By comparison, Applicants' claimed method has a significant advantage over Wild by allowing the determination of birth dates. See, pending claims 17, 21-23, 32 and 33. As discussed above, Applicants' claimed invention allows the determination of birth dates to within 5 years of the actual birth.

17. As discussed above, Wild does not disclose the use of tooth enamel, the addition of Hedges does not cure the deficiencies of Wild because there is no motivation to combine the two references. Hedges' method is different from Wild and the two methods cannot be combined because they are incompatible. Hedges is directed to a method of dating ancient samples by treating samples with (1) H_2O_2 and (2) HAc under vacuum and collecting and dating CO_2 which evolves under HAc (See, Hedges, page 287, page 287, 2nd to 4th paragraph).

18. Unlike Applicants' invention as recited in the instant claims, Hedges never determined a birth date to within 5 years of actual birth for any of the tooth enamel samples investigated. The determination of a birth date of any of Hedges' samples to within 5 years, as claimed in the pending claims, would be impossible using Hedges' method because Hedges' method had errors of between 80 to 1700 years (See Hedges, Table 1). Hedges provide no disclosure or suggestions on how the error rates can be reduced from 80-1700 years as disclosed in Hedges to 5 years as in the claimed invention.

19. It is my opinion that a person of skill in the art would find no motivation to combined the samples of Hedges with the methods of Wild and Hedges. That is because a person of skill in the art would understand that Wild and Hedges have contradicting and incompatible requirements for reagents. Wild requires acetone soluable lipids for determination of a day of death. See, Wild, page 274, section under "sample preparation". In contrast Hedges requires enamel to be treated under (1) H_2O_2 and (2) HAc under vacuum for CO_2 collection. (See, Hedges, page 287, page 287, 2nd to 4th paragraph). Hedges' bone enamel is not soluble in acetone and Wild's lipids cannot survive acid and base treatment. For these reasons, Wild's method is incompatible with Hedges' method and a person of skill in the art would find no motivation to combine the two methods.

20. It is also my opinion that a person of skill in the art would realize that a combination of Wild and Hedges would lead to a method that is not functional.

21. If Hedges' enamel is used in the method of Wild, there is no possibility of success because Wild is based on detection of ^{14}C in acetone soluable lipids and enamel does not have lipids. See, Wild, page 274, section under "sample preparation". Enamel is composed of apatite, a compound that is insoluable in acetone. Wild's method relies on acetone to extract lipids from tissue samples. Since enamel is insoluable, Wild's method cannot extract any meaningful carbon for dating purposes. For this reason, a combination of

Wild and Hedges would not lead to any data indicating a "birth date" - as claimed in the instant claims.

22. In addition, even if Wild and Hedges were combined (for the sake of argument since I believe that they cannot be combined to become a functioning method) the combination would not lead to Applicants' claimed invention.

23. One significant advantage of the claimed invention is that it allows the determination of birth dates - a process that was not possible until the instant claimed invention. Traditional method of ^{14}C dating cannot determine the birth date of organism. The methods of the invention therefore fulfill a long-felt need for rapid determination of birth dates. Therefore, the claimed invention is directed to a process entirely different in its steps and results than any other study to date. All studies performed before the claimed invention, including those of Hedges and Wild, used carbon dating to determine tissue age (i.e., time of death of a tissue). Before Applicants' claimed invention, there has been no attempt to use carbon dating and the bomb-spike to determine the *birth date* of an animal.

24. While Wild and Hedges have used biological samples to determine a date of death, neither Wild nor Hedges has attempted to determine the age of an animal from biological samples - a process that is claimed in the instant claims. All carbon-14 dating thus far has been done to either determine (i) the age of a fossil (for archaeological samples) or (ii) the date of death of a specific tissue (for modern samples). None of these techniques can provide the *"birth date"* within an accuracy of *5 years* - recitations of the instant claims. For at least this reason, the claimed invention is novel and nonobvious over the cited references.

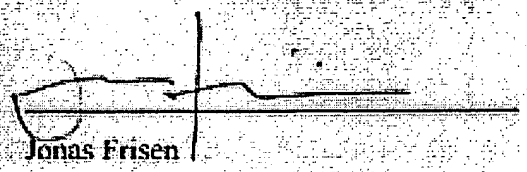
25. In summary, the claimed invention is directed to a process entirely different in its steps and results than any other study to date. All studies performed before the claimed invention, including those of Hedges and Wild, used carbon dating to determine tissue age (i.e., time of death of a tissue). Before Applicants' claimed invention, there has been no attempt to use carbon dating

and the bomb-spike to determine the birth date of an animal. The fact that this has never been attempted, and proof that our work is novel and important, has been acknowledged by our peers. This is evident from the publication of our work following peer review in Nature (Spalding et al., 2005 Nature vol 437:333-334. Exhibit 1).

26. Finally, we have received praise for the claimed method. Our publication of the methods of the claimed invention (described in the previous paragraph) was described by SEED magazine (SEED media group, NY, USA; Dec/Jan 2005, pg 88) as "one of the 10 most revelatory experiments, findings, discoveries and proofs of the year" for 2005 (Enclosed as Exhibit 2).

27. For the reasons stated above, it is my opinion that the claimed methods, the determination of a birth date based on tooth enamel analysis, represent a significant advancement in the field which is novel and which has never been successfully performed before. The claimed method is thus both novel and not obvious over a combination of Wild and Hedges (if the combination were proper, which in my opinion it is not).

28. I declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.



Jonas Frisen

Signed this day 19 of September, 2006

BRIEF COMMUNICATIONS

Age written in teeth by nuclear tests

A legacy from above-ground testing provides a precise indicator of the year in which a person was born.

Establishing the age at death of individuals is an important step in their identification and can be done with high precision up to adolescence by analysis of dentition, but it is more difficult in adults. Here we show that the amount of radiocarbon present in tooth enamel as a result of nuclear bomb testing during 1955–63 is a remarkably accurate indicator of when a person was born. Age is determined to within 1.6 years, whereas the commonly used morphological evaluation of skeletal remains and tooth wear is sensitive to within 5–10 years in adults.

The amount of carbon-14 isotope (^{14}C) in the atmosphere remained relatively stable until 1955, when above-ground nuclear bomb tests caused it to rise dramatically[1,2]. Although the bombs were detonated at only a few locations, the additional ^{14}C in the atmosphere rapidly equalized around the globe. Since the Test Ban Treaty in 1963, atmospheric ^{14}C has been dropping exponentially (Fig. 1a). This is not primarily because of radioactive decay (the half-life of ^{14}C is 5,730 years) — it is also due to diffusion from the atmosphere[3]. Atmospheric ^{14}C reacts with oxygen to form carbon dioxide, which is incorporated into plants by photosynthesis; by eating plants, and animals that feed on plants, the ^{14}C concentration in the human body closely parallels that in the atmosphere at any given time[4–6].

The enamel of individual teeth is formed at distinct, well characterized times during childhood[7,8] and it contains 0.4% carbon. There is no turnover of enamel after it has been laid down, so the ^{14}C concentration reflects that in the atmosphere at the time of enamel formation. We measured the ^{14}C content of tooth enamel (for methods, see supplementary information) and related it to the known concentrations in the atmosphere in different years to establish the year of tooth formation. This date was then related to the known age for enamel deposition of individual teeth[7] to establish the person's year of birth (Fig. 1a).

We found that this method gave a remarkably precise estimate of age for 22 individuals ($R^2 = 0.99$ from regression shown in Fig. 1b; for details, see supplementary information). The average systematic deviation from the correct value was +0.2 years, and the average absolute error for individual measurements was 1.6 ± 1.3 years (s.d.). This indicates that the precision is substantially higher than that obtained by other available methods[9].

The final formation of enamel is for the wisdom teeth at 12 years of age. For individuals born before 1943 (12 years before the onset of nuclear bomb testing), we can therefore conclude by this method only that birth occurred before that year, albeit with a high degree of certainty (100% correct in our analysis (Fig. 1b); $n = 4$). In any case of ambiguity as to whether birth occurred before or after the peak of nuclear bomb testing, it is necessary to analyse two teeth that were formed at different ages: this distinguishes whether the ^{14}C measurements relate to the rising or falling part of the ^{14}C curve (Fig. 1a).

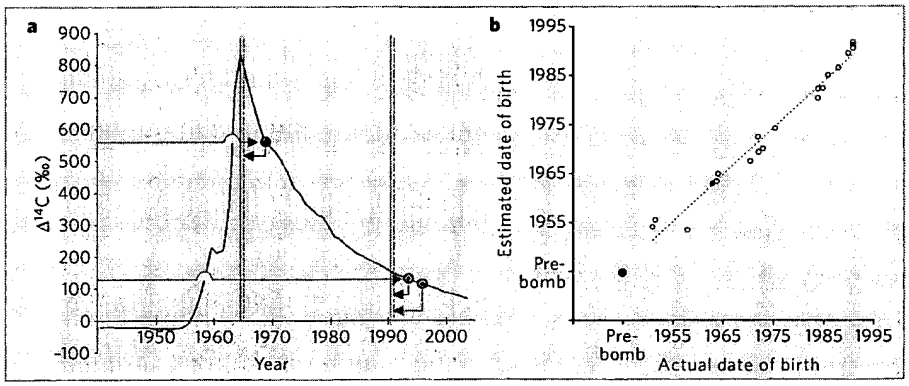


Figure 1 | Date of birth determined from ^{14}C in teeth. **a,** Nuclear bomb tests during 1955–63 produced large amounts of ^{14}C, which have since declined exponentially (blue line). The ^{14}C:C ratio has ranged from 1.15×10^{-12} to 2.20×10^{-12} since 1950. $\Delta^{14}C$ represents the ^{14}C value corrected for radioactive decay and ^{13}C fractionation (see supplementary information). To estimate an individual's date of birth, the ^{14}C concentration measured in their tooth enamel is plotted on to the curve of atmospheric $\Delta^{14}C$ against time (blue) to find the year of enamel synthesis (right-pointing arrows), and the known age at enamel formation for individual teeth was then subtracted from the year obtained to give the date of birth (left-pointing arrows; dashed vertical lines). Two representative cases are shown (red and green); two teeth were analysed for the case depicted in green. Solid vertical lines, actual dates of birth. **b,** Relation between estimated and actual dates of birth. Each point corresponds to one individual, except for the 'pre-bomb' point, which represents four individuals; coloured points are cases shown in **a.**

The sensitivity of our method is mainly determined by variation between individuals in their age at tooth formation, and the precision of the ^{14}C measurement. The degree of inter-individual variation is different for different teeth, so selection for ^{14}C measurement of teeth with the least variation[8] and of several teeth from the same individual should give a more accurate date of birth. With regard to measurement precision, we cannot exclude the possibility that differences in diet or in local conditions might contribute some variability in the amount of ^{14}C incorporated into tooth enamel. Although such an effect is not supported by results from comparative analyses of different foodstuffs produced in rural and industrial areas[10], the method will need to be verified on a larger number, and perhaps on a wider geographical range, of cases before it can be applied to forensic work.

Although the nuclear bomb tests were conducted several decades ago and the resulting change in atmospheric ^{14}C is now decreasing only slowly (Fig. 1a), the method described here should allow precise age determination for a long time to come because techniques for ^{14}C measurement are becoming increasingly sensitive. In addition, accelerator mass spectrometry for ^{14}C analysis has become more accessible and inexpensive, making the potential application of our dating method no more difficult than other methods now used in routine forensic examinations.

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1. De Vries, H. *Science* **128,** 250–251 (1958).
2. Nydal, R. & Lovseth, K. *Nature* **206,** 1029–1031 (1965).
3. Levin, I. & Kromer, B. *Radiocarbon* **46,** 1261–1272 (2004).
4. Libby, W. F., Berger, R., Mead, J. F., Alexander, G. V. &

Ross, J. F. *Science* **146,** 1170-1172 (1964).
5. Harkness, D. D. *Nature* **240,** 302-303 (1972).
6. Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H. & Frisén, J. *Cell* **122,** 133-143 (2005).
7. Nolla, C. M. *J. Dent. Child.* **27,** 254-266 (1960).
8. Bolanos, M. V., Manrique, M. C., Bolanos, M. J. & Briones, M. T. *Foren. Sci. Int.* **110,** 97-106 (2000).
9. Ritz-Timme, S. *et al. Int. J. Legal Med.* **113,** 129-136 (2000).
10. Otlet, R. L., Walker, A. J., Fulker, M. J. & Collins, C. *J. Envir. Radioact.* **34,** 91-101 (1997).

Supplementary information accompanies this communication on *Nature*'s website.
Competing financial interests: declared (see online version of the communication).
doi:10.1038/437333a

BOTANY

Floral fluorescence effect

The way flowers appear to insects is crucial for pollination[1-3]. Here we describe an internal light-filtering effect in the flowers of *Mirabilis jalapa*, in which the visible fluorescence emitted by one pigment, a yellow betaxanthin, is absorbed by another, a violet betacyanin, to create a contrasting fluorescent pattern on the flower's petals. This finding opens up new possibilities for pollinator perception as fluorescence has not previously been considered as a potential signal in flowers.

We investigated the spectra and distribution of the pigments in the multicoloured, strikingly patterned flowers of *M. jalapa* (Nyctaginaceae), which open only in the late afternoon. This and related plants, such as *Bougainvillea*, *Celosia*, *Gomphrena* and *Portulaca*, contain pigments known as betalains. These comprise the yellow, fluorescent betaxanthins[4] and violet betacyanins, of which betanin (betanidin-*O*-β-glucoside) is the most common.

We extracted and purified the pigments of *M. jalapa* flowers and analysed them by high-performance liquid chromatography, as previously described[5]. The analysis confirmed that the pigmentation pattern on the flowers was due to a mixture of betaxanthins and betanins. Measurement of the fluorescence-emission spectrum of dopaxanthin and the absorbance spectrum of betanin indicates that the light emitted by the fluorophore is strongly re-absorbed (Fig. 1). Addition of increasing concentrations of betanin to the dopaxanthin solution reduced the intensity of its fluorescence, until only 30% of the initial fluorescence was detectable at a ratio of 8.5:1. (For details and methods, see supplementary information.)

This internal light-filtering effect between the two types of betalain plant pigment causes a fading of visible fluorescence on parts of the flower where both types are present; areas containing only betaxanthins appear yellow under white light because of a combination of fluorescence and reflectance of non-absorbed radiation (Fig. 2a). The effect can be demonstrated in a system designed to visualize green fluorescence, which filters the incident light to blue and causes betaxanthins in the flower to fluoresce by emitting green light (Fig. 2b).

Detailed images of different zones of petal coloration were obtained by using light and fluorescence microscopy. A brightfield image under white light shows some cells containing only betaxanthins (Fig. 2c, yellow), others with betacyanins (Fig. 2c, deep-red spots), and some with both pigments together (Fig. 2c, orange).

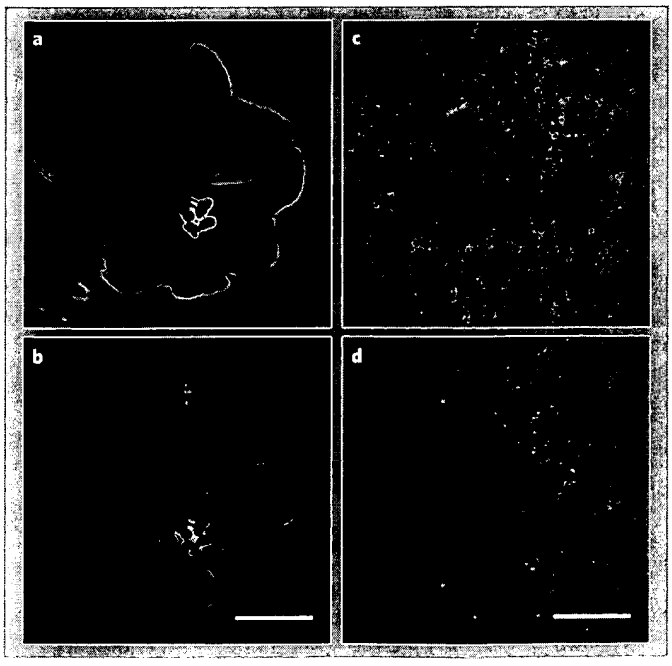

Figure 2 | Visible fluorescence in *Mirabilis jalapa* petals. **a, b,** Flower with areas of red or yellow coloration under white light (**a**); only the yellow areas emit green fluorescence when excited by blue light (**b**) (scale bar, 1.5 cm). **c, d,** Light micrographs of a section of a single red-and-yellow petal, showing brightfield (**c**) and fluorescent (**d**; excitation wavelength, 450–490 nm) images (scale bar, 500 μm). Green fluorescence is due to betaxanthins; dark areas correspond to orange areas in **c**, where light emitted from the fluorescent pigment is absorbed by betanin.

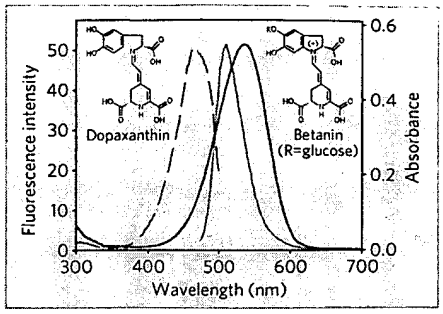

Figure 1 | Spectra of dopaxanthin and betanin. Dopaxanthin is used as a model betaxanthin because of its structural (insets) and biochemical similarity to betacyanins. When excited by blue light, betaxanthins emit green fluorescence[4]. Fluorescence spectra (blue line, excitation spectrum; green line, emission spectrum) for natural dopaxanthin (6.0 μM, in water) are shown; violet line, absorbance spectrum of pure betanin (8.4 μM, in water). Note the overlap of the emission and absorbance spectra of the pigments.

The fluorescence micrograph shows that fluorescence is inhibited in areas where betaxanthins coexist with betanin (Fig. 2d) — the dark area corresponds to the orange area in Fig. 2c.

Fluorescence can be an important signal in mate choice for budgerigars[6] and possibly in mantis shrimp[7], and it may be that in flowers it attracts pollinators. The patterns arising from the internal light-filtering effect between betalain pigments described here could encourage bees[1] and bats[8], which have visual receptors that are sensitive to green light and can detect bright targets better than dim ones[9]. Variation in light emission by flowers at visible wavelengths also modifies their colour, which would enhance their visibility to pollinators[10].

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1. Gumbert, A. *Behav. Ecol. Sociobiol.* **48,** 36-43 (2000).
2. Giurfa, M., Eichmann, B. & Menzel, R. *Nature* **382,** 458-461 (1996).
3. Heiling, A. M., Herberstein, M. E. & Chittka, L. *Nature* **421,** 334 (2003).
4. Gandía-Herrero, F., García-Carmona, F. & Escribano, J. *J. Chromatogr. A* **1078,** 83-89 (2005).
5. Gandía-Herrero, F., Escribano, J. & García-Carmona, F. *Plant Physiol.* **138,** 421-432 (2005).
6. Arnold, K. E., Owens, I. P. F. & Marshall, N. J. *Science* **295,** 92 (2002).
7. Mazel, C. H., Cronin, T. W., Caldwell, R. L. & Marshall, N. J. *Science* **303,** 51 (2004).
8. Winter, Y., Lopez, J. & von Helversen, O. *Nature* **425,** 612-614 (2003).
9. De Ibarra, N. H., Vorobyev, M., Brandt, R. & Giurfa, M. *J. Exp. Biol.* **203,** 3289-3298 (2000).
10. Vorobyev, M., Marshall, J., Osorio, D., De Ibarra, N. H. & Menzel, R. *Color Res. Appl.* **26** (suppl.), 214-217 (2001).

Supplementary information accompanies this communication on *Nature*'s website.
Competing financial interests: declared none.
doi:10.1038/437334a

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WRAP-UP

Year in Science: Ideas

Seed presents the ten most revelatory experiments, findings, discoveries and proofs of the year. Welcome to the new scientific renaissance.

by • Posted December 27, 2005 02:46 PM

From the DEC/JAN 2006 issue of Seed:

CHEMICALS CAN CURE ADDICTION

Credit: Elizabeth Huey

With health and social costs of more than $500 billion a year, drug and alcohol addiction is among the most damaging diseases in the U.S. While neuroscience has made great strides in understanding the basis of addiction (it involves floods of dopamine), that knowledge has yet to yield practical results.

This is beginning to change. Two papers published in Neuron show that addiction—at least in rats—is a treatable medical problem. Each took an unconventional approach, focusing not on the addictive urge itself but on disrupting the memory of the addiction, the theory being that you can't be addicted to that which you can't remember.

The first study, by John Marshall and Courtney Miller of the University of California at Irvine,
attempted to eradicate the memories of rodents with a taste for cocaine. After being administered a drug that inhibits production of the protein extracellular signal-regulated kinase, the rats forgot in which cage they received their fix. The unfortunate rodents had gone clean, and they didn't even need 12 steps.

The second study, by Dr. Jonathan Lee of Cambridge University, focused on the amygdala, the part of the brain that learns to pair the pleasurable feeling of intoxication with a certain stimulus (say, the neon sign of a favorite bar or the sight of an ashtray). He wanted to see whether turning off a particular neuronal

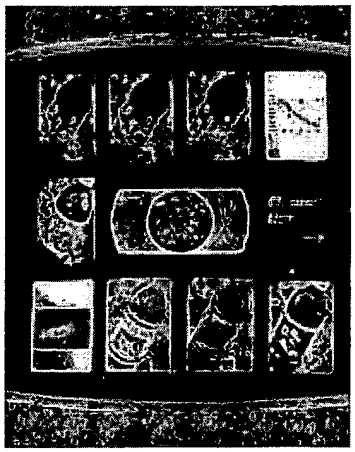
HUMAN CLONING IS MORE THAN JUST A HYPOTHETICAL

The headline was alarming: "South Korea Makes Strides in Human Cloning" The reality was not so Huxleyan. Dr. Woo Suk Hwang's lab had come up with an extremely efficient method of producing stem cells. First, they created embryos that were exact genetic matches of individuals. After letting the embryo divide a few times, they extracted its stem cells. This method—known as therapeutic cloning—is one of the most promising approaches in the stem-cell field. Researchers believe that therapeutic cloning will one day be used to create replacement tissues for a variety of diseases. Hwang made it look easy.

Three months later, Hwang made headlines again: After working for more than 900 consecutive days, his lab announced they had cloned man's best friend, the dog (an Afghan hound named Snuppy). At first glance, that may not seem like such a big deal; after all, scientists cloned Dolly the sheep a decade ago. But for a variety of reasons, dogs had been notoriously difficult to clone. (Dogs ovulate unpredictably, their eggs require surgery to extract, and their embryos won't grow outside the uterus.) Nevertheless, Hwang says, "Dogs can be good models for human diseases. Dogs [can contract] more than 50 diseases that are similar to human diseases."

The fear is that Hwang's techniques may open the door to human cloning before the many ethical issues it raises have been adequately addressed. Hwang himself is unapologetic. Regardless, his technical brilliance showed that it's no longer just a theoretical possibility.

AT THE DAWN OF TIME, THERE WAS A PERFECT LIQUID



Credit: Brian Ulrich

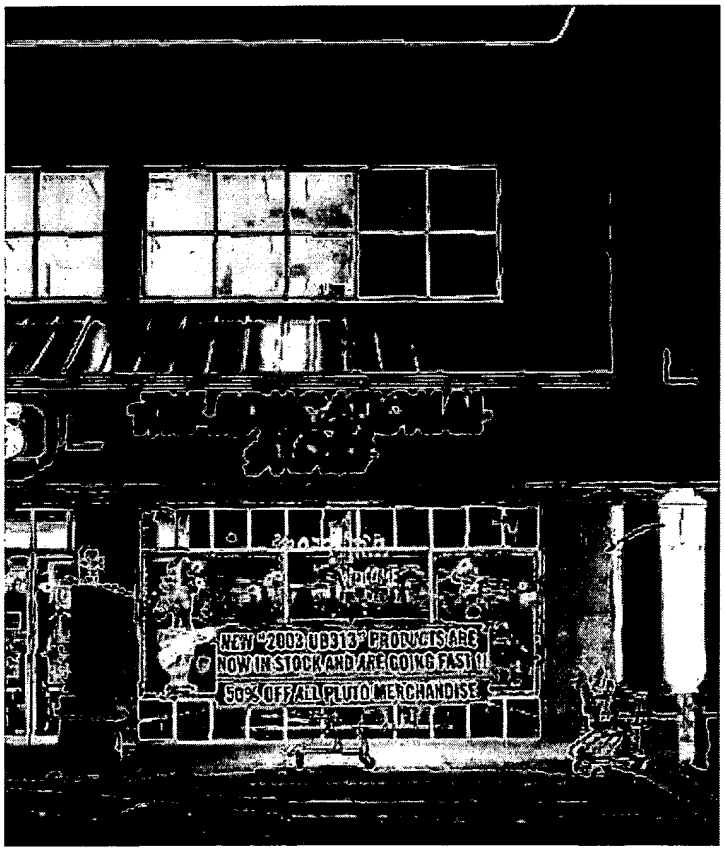
In the timeless time after the big bang, when the universe was as new as it was empty, there were only quarks and gluons. Atoms had to yet to coalesce. But what was this proto-universe like? In the nanosecond before there was matter, what was there?

There was a perfect liquid. Experiments at the Relativistic Heavy Ion Collider (RHIC) at the Brookhaven National Laboratory have demonstrated that quarks and gluons, when freed from their workaday reality as the building blocks of nuclei, become a liquid without viscosity, a fluid

RHIC proved this by accelerating gold nuclei to 99.7% the speed of light (RHIC scientists actually believe it was 99.9%, but can only conclusively establish the average of "closer to 99.7%," according to a spokesman—a difference of 600,000 meters per second), then smashing them together in an explosion so powerful it generated temperatures close to those of the big bang. An interpretation of the resulting atomic debris—which disappeared in less than a hundredth of a billionth of a trillionth of a second—showed the clear presence of a liquid.

To most, this was a complete surprise. After all, things that are a trillion degrees hot are normally gases or plasmas, not frictionless liquids. But not all physicists were so flabbergasted. String theorists—hitherto best known for believing in the existence of 10 dimensions—have argued in their own work that the big bang immediately gave way to a sloshy quark-gluon soup. The Brookhaven data tenuously confirms their expectations.

"PLANET" NEEDS A NEW DEFINITION



has a faithful moon and is significantly larger in diameter than Pluto. It is the most distant object ever seen orbiting the sun. It is 2003 UB313, and it resides way out there in the Kuiper belt, a vast swath of icy bodies somewhere beyond Neptune.

Caltech astronomer Dr. Mike Brown, who discovered UB313, calls the Kuiper belt "a fossil record of the solar system. Essentially, the objects in the Kuiper belt have been in deep freeze for 4.5 billion years. Things haven't changed much. So the more we know about the Kuiper belt, the more we know about the formation of our solar system."

But is it really a planet? The question has dogged Brown and his team since they first glimpsed UB313. "I personally wouldn't call it a planet," says Brown. "I only think there are eight planets. If I had my way, I would call UB313 and Pluto Kuiper belt objects, not planets. Of course, they are very significant Kuiper belt objects."

HUMANS REALLY ARE 98% CHIMP

Credit: Ben Fry

It's now a fact: We are just hairless chimps. In September, the first analytical comparisons of the chimpanzee and human genomes—led by teams at MIT, Harvard and Washington University School of Medicine, in St. Louis—were published in *Nature*. The results were unsettling: man is 98.77% chimpanzee (the 98% figure tossed around for years was really just an approximation). In fact, since chimps and humans parted ways on the evolutionary chart about six million years ago, most proteins of *Homo sapiens* have accumulated a grand total of one unique change.

most uniquely human part of the body is not the brain, but the testes. The study's lead author, Tarjei Mikkelsen, isn't surprised: "This finding actually makes sense. Sexual selection is a very prominent force in primates. A big guy with the fastest swimming sperm will have the most kids."

Of course, humans are not merely chimps with sprightly sperm. The human brain underwent a rapid anatomical renaissance, all because of a few flicks of the right developmental switch. The study revealed that natural selection did not go about redesigning us base pair by base pair; that would have taken way too long. Rather, by changing how our genes are expressed—i.e., altering the molecules, called transcription factors, that regulate the activity of other genes—natural selection achieved profound effects with minimal rewriting.

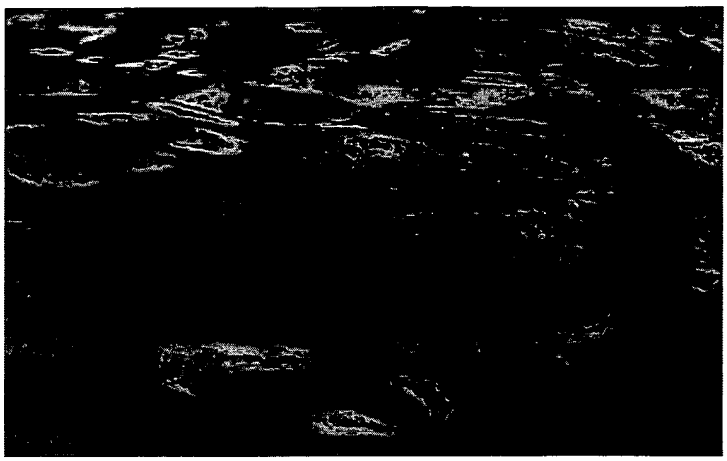
HOBBITS "LIVED"

It sounds like a lost chapter of *The Lord of the Rings*: On a remote tropical island, there lived a species of tiny people, one meter tall, with skulls the size of grapefruits, who hunted Komodo dragons, dwarf elephants and giant rats.

But this is a true story, a finding that completely recasts the range of morphological adaptation and variation for the genus *Homo*. An archaeological team led by Dr. Peter Brown, of the University of New England in Australia, discovered the remains of this new human species last year on Flores, an island 600km from Bali. More bones were unearthed this year, confirming, for most, Brown's initial interpretation that this was a new species and not a microencephalic or dwarf *Homo sapiens*.

"When I first saw the skeleton," said Brown, "I was speechless. The last time people of this brain size and body size walked the planet was millions of years ago, in Africa. What was it doing on Flores? I would have been less surprised if the team had unearthed an alien spacecraft." Stranger yet is the age of the skeleton. The little lady was less than 20,000 years old, which means that *Homo floresiensis* shared the earth with *Homo sapiens*. This has sent researchers scrambling to rethink the story of human evolution. "Humans are just another mammal," said Brown. "I'm happy to see a demonstration that they appear to follow the same evolutionary processes on islands as other large-bodied

GLOBAL WARMING CAUSES STRONGER HURRICANES



Credit: Gregory Ryan

Global warming is no longer the apocalypse of the distant future. Last summer, the world learned just how devastating a few degrees of greenhouse warmth can be. Two monstrous hurricanes barreled into the Gulf Coast, and the result was devastating: The ocean swallowed a city.

Global warming does not cause hurricanes. Butterflies flapping their wings in Tokyo cause hurricanes. But three papers, using different statistical approaches, demonstrated the strong correlation between global warming and hurricane intensity. Global warming makes hurricanes worse.

The theory is simple: Rising atmospheric temperatures lead to warmer ocean surface temperatures, causing more water to evaporate, which gives storms more water and energy. The Gulf of Mexico is 2.8°C (5°F) warmer than "normal" this year.

The strongest case was made by Kerry Emanuel, a meteorologist at MIT. "What the data shows," Emanuel says, "is that a measure of the total amount of power generated by hurricanes globally over the past 30 to 50 years has increased by 70 to 80%. That's a really big increase." Although some have cautioned against attributing the power of any hurricane to a single cause, the correlation showed by Emanuel and others, and the images of destruction along the Gulf Coast, have indelibly changed the discourse on climate change.

RADIOACTIVE DECAY DATES HUMAN CELLS

In the July issue of *Cell*, Dr. Jonas Frisen, a biologist at the Karolinska Institute in Stockholm, announced a method for the dating of human cells. His stopwatch is the fallout of the nuclear age.

From 1955 to 1963, thousands of atomic weapons were detonated, dispersing carbon-14 all over the earth. Since carbon-14 decays at a regular rate, Frisen's creative insight was that the amount of carbon-14 contained in the DNA of a cell, being closely correlated with the amount of carbon-14 in the atmosphere at the time of the cell's birth, could be used to calculate the age of that cell.

It took Frisen and his team four years experimenting on Swedish pine trees and dead horses to perfect the technique, but the hypothesis played out perfectly. Frisen's lab began dating cells throughout the body. Intestinal cells, they found, regenerate about every 15 years. And while the cerebellum and the visual cortex are about the same age as the individual (they don't die until you do), preliminary results suggest that other parts of the brain—such as the hippocampus—undergo constant cellular division. Evidently, your self is always reinventing itself.

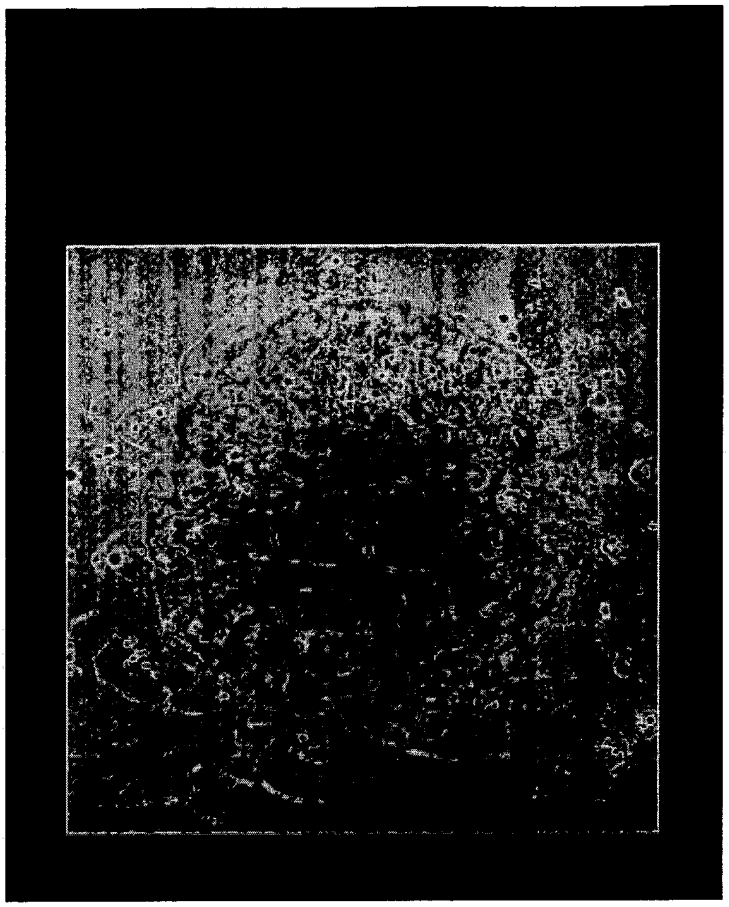
Frisen's notion has an intriguing application: forensics. Frisen's lab realized that the carbon-14 in tooth enamel can help determine the age of a corpse within 1.6 years' accuracy. This method has already been used to identify victims of last year's Indian Ocean tsunami and is currently helping identify victims of Hurricane Katrina and its aftermath.

STEM CELLS CAN BE MADE FROM ADULT CELLS

A stem cell is a tabula rasa: a living form capable of taking any shape. We, however, are composed almost entirely of adult cells. Our cells have differentiated; their genes have instructed them to become something specific. Until now, no one thought skin could change its mind and go back to become a neuron.

But nothing in life is irrevocable. Harvard biologists led by Kevin Eggan and Chad Cowan made an adult cell turn back into a blank slate. "Our experiment was relatively simple," says Eggan. "We took a human skin cell, fused it with an

The experiment's success has intriguing potential. Eggan cautions his that technique "is complementary to, not a replacement for, research involving embryos—after all, we could never have done this experiment [without] access to embryonic stem cells." But if the Harvard fusion approach is perfected—it might take a while—will scientists be able to harvest stem cells without using embryos, thus side-stepping the political controversy? Eggan says this is a real possibility.



Credit: Ariel Ruiz i Altaba

INFORMATION CAN ESCAPE FROM BLACK HOLES

The fate of information hangs on this question: Do black holes destroy everything they inhale? Stephen Hawking once demonstrated that the radiation emitted by black holes evaporates them. Then in 1976, he published a paper arguing that black holes not only disappear, but all the information they contain—and they can vacuum a galaxy—disappears with them. Poof.

Hawking's theory was bad news for physics. According to quantum mechanics, information is impossible to destroy. Burn a book and, at least in theory, its text is

As a result, physics is ultimately limited in what it can know.

For 20 years, the "information paradox" seemed unavoidable. That all changed in 1997, when Juan Maldacena—an Argentinian research mathematician at Harvard—announced that he had found the "lost" information. It had been hiding in "an equivalent description of reality," a "holographic" representation that was implied by the laws of negatively curved space.

Despite the surreal elegance of Maldacena's proof, Hawking denied its implications. Then, last summer, he revealed the groundwork for a proof consistent with Maldacena's conclusion that black holes do not destroy their information. Knowledge—even obliterated by the gravity of black holes—stubbornly survives.

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Year in Science: Ideas, written by , posted on December 27, 2005 02:46 PM, is in the category Wrap-Up. Permalink.